

CLEAN VERSION OF AMENDMENTS

IN THE SPECIFICATION

The paragraph on page 11, line 38 to page 12, line 2, has been amended to read as follows:

For example, the plant expression cassette can be incorporated into the tobacco transformation vector pBinAR-Hyg. Fig. 2 shows the tobacco transformation vectors pBinAR-Hyg with the 35S promoter (A) and pBinAR-Hyg with the seed-specific promoter phaseolin 796 (B):

- HPT: hygromycin phosphotransferase
- OCS: octopine synthase terminator
- PNOS: nopaline synthase promoter
- also drawn in are those restriction cleavage sites which cut the vector only once.

The paragraph on page 31, lines 5-24, has been amended to read as follows:

Oligonucleotides for a PCR were derived from the DOXS DNA sequence (Acc. Number AF035440), and a BamHI restriction cleavage site was attached to them at the 5' end, and an XbaI or another BamHI restriction cleavage site was attached to them at the 3' end. The oligonucleotide at the 5' end comprises the sequence 5'-atggatccat gagtttt-gat attgccaaat ac- 3' (SEQ ID No. 9) (nucleotides 1-24 of the DNA sequence; in italics) starting with

the ATG start codon of the gene, and the oligonucleotide at the 3' end comprises the sequence 5'- *attctagatt atgccagcca ggccttg-* 3' (SEQ ID No. 10) or 5'- *atggatcctt atgccagcca ggccttg-* 3' (SEQ ID No. 11) (nucleotides 1845-1863 of the reverse complementary DNA sequence; in italics) starting with the stop codon of the gene. The PCR reaction with the two BamHI-containing oligonucleotides was carried out with Pfu polymerase (Stratagene GmbH, Heidelberg) in accordance with the manufacturer's information. 500 ng of the genomic DNA from *E. coli* were employed as template. The PCR program was as follows:

5 cycles: 4 sec 94°C, 30 sec 52°C, 2 min 72°C;
5 cycles: 4 sec 94°C, 30 sec 48°C, 2 min 72°C;
25 cycles: 4 sec 94°C, 30 sec 44°C, 2 min 72°C.

Amend the paragraph on page 36, lines 11-20, as follows:

Oligonucleotides were derived for a PCR from the DNA sequence of the HPPD from *Streptomyces avermitilis* (Denoya et al., 1944; Acc. Number U11864), and a BamHI restriction cleavage site was attached to the 5' end of them and an XbaI restriction cleavage site was attached at the 3' end of them. The oligonucleotide at the 5' end comprises the sequence 5'- *ggatccagcg gacaagccaa c-* 3' (SEQ ID No. 12) (37 to 55 bases distant from the ATG in the 5' direction; in italics), and the oligonucleotide at the 3' end comprises the sequence 5'- *tctagattatgccagccaggccttg-* 3' (SEQ ID

No. 13) (nucleotides 1845-1863 of the reverse complementary DNA sequence; in italics).

Amend the paragraph on page 37, lines 28-44, as follows:

To clone the HPPD in vectors which additionally contain another cDNA, oligonucleotides were derived for a PCR and had a BamHI restriction cleavage site attached at the 5' end and at the 3' end. The oligonucleotide at the 5' end comprises the sequence 5'-*ggatcctcca gcggacaagc caac*- 3' (SEQ ID No. 14) (nucleotides 37 to 55 distant from the ATG in the 5' direction; in italics), and the oligonucleotide at the 3' end comprises the sequence 5'-*atggatcccg cgccgcctac aggttg*- 3' (SEQ ID No. 15) (terminating at base pair 1140 of the coding sequence, starting 8 base pairs 3' from the TAG stop codon; in italics). The PCR reaction was carried out with Tli polymerase (Promega GmbH, Mannheim) in accordance with the manufacturer's information. 10 ng of the plasmid pBinAR-HPPD were employed as template. The PCR program was as follows:

5 cycles: 94°C 4 sec, 68°C 30 sec, 72°C 2 min;
5 cycles: 94°C 4 sec, 64°C 30 sec, 72°C 2 min;
25 cycles: 94°C 4 sec, 60°C 30 sec, 72°C 2 min.

Amend the paragraph on page 38, lines 11-28, as follows:

For the cloning, the 35S promoter, the transketolase transit peptide, the HPPD gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al. 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-HPPD by PCR. A HindIII cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the 5' region of the promoter (in italics) is 5'- *ataagcttca tggagtcaaa -gattcaaata ga*- 3' (SEQ ID No. 16), and that of the oligonucleotide which anneals onto the termination sequence (in italics) is 5'- *ataagcttgg acaatcagta aattgaacgg ag* -3' (SEQ ID No. 17). The resulting fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred as HindIII fragment from this PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984, Nucleic Acids Res. 12, 8711-8721).

Amend the paragraph on page 38, lines 30-46, as follows:

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated by PCR from the plasmid pBinAR-TP-

DOXS. An EcoRI cleavage site was attached to each of the oligonucleotides for the promoter and terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'- *atgaattcca tggagtcaaa gattcaaata ga* -3' (SEQ ID No. 18), and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'- *atgaattcgg acaatcagta aattgaa-cgg ag* -3' (SEQ ID No. 19). The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script (Stratagene GmbH, Heidelberg). The correctness of the sequence was checked by sequencing (SEQ ID No. 3). It was transferred as EcoRI fragment from the PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984).

Amend the paragraph on page 42, lines 17-26, as follows:

Oligonucleotides were derived for a PCR from the geranylgeranyl-pyrophosphate oxidoreductase DNA sequence (Keller et al., Eur. J. Biochem. (1998)251(1-2), 413-417; Accession Number Y14044), and a BamHI restriction cleavage site was attached at the 5' end of these and a SalI restriction cleavage site was attached at the 3' end. The oligonucleotide at the 5' end comprises the sequence 5'- *atggatccat ggcgacgacg gttacactc* -3' (SEQ ID No. 20) starting with the first codon of the cDNA (in italics), and the oligonucleotide at the 3' end comprises the sequence 5'-*atgtcgacgt gatgatagat*

tactaacaga c -3` (SEQ ID No. 21) starting with base pair 1494 of the cDNA sequence (in italics).

Amend the paragraph on page 43, line 34, to page 44, line 3, as follows:

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-DOXS by PCR. An EcoRI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'- *atgaattcca tggagtcaaa gattcaaata ga -3`* (SEQ ID No. 22), and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'- *atgaattcgg acaatcagta aattgaacgg ag -3`* (SEQ ID No. 23). The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as EcoRI fragment into the correspondingly cut vector pBin19 (Bevan, Nucleic Acids Res. 12 (1984), 8711-8721).

Amend the paragraph on page 44, lines 5-32, as follows:

The 35S promoter, the GGPPOR gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinARHyg-GGPPOR by PCR. An XbaI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'- *attctagaca tggagtcaaa -gattcaaata ga* -3' (SEQ ID No. 24), and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'- *attctagagg acaa-tcagta aattgaacgg ag* -3' (SEQ ID No. 25). The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information onto the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as XbaI fragment into the correspondingly cut vector which already contained, as described above, the DOXS sequence. The result was the construct pBinAR-DOXS-GGPPOR (Figure 15), whose fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment B comprises the transit peptide of the plastidic transketolase. Fragment E comprises the DOXS gene. Fragment D

comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment F comprises the GGPPOR gene including the intrinsic plastid transit sequence.

Amend the paragraph on page 45, lines 1-17, as follows:

To clone HPPD into vectors which additionally contain another cDNA, oligonucleotides were derived for a PCR, and a BamHI restriction cleavage site was attached to them at the 5' end and 3' end. The oligonucleotide at the 5' end comprises the sequence 5'- *ggatcctcca gcggaacaagc caac* -3' (SEQ ID No. 26) (nucleotides 37 to 55 distant from ATG in the 5' direction; in italics), and the oligonucleotide at the 3' end comprises the sequence 5'- *atggatcccg cgccgcctac aggttg* -3' (SEQ ID No. 27) (ending with base pair 1140 of the coding sequence, starting 8 base pairs 3' of the TAG stop codon; in italics). The PCR reaction was carried out with Tli polymerase from Promega GmbH, Mannheim in accordance with the manufacturer's information. 10 ng of the plasmid pBinAR-HPPD were employed as template. The PCR program was as follows:

5 cycles: 94°C 4 sec, 68°C 30 sec, 72°C 2 min;
5 cycles: 94°C 4 sec, 64°C 30 sec, 72°C 2 min;
25 cycles: 94°C 4 sec, 60°C 30 sec, 72°C 2 min.

Amend the paragraph on page 45, lines 29-46, as follows:

For the cloning, the 35S promoter, the transketolase transit peptide, the p-HPPD gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al. 1984) for termination of transcription was isolated from the plasmid pBinAR-TP-p-HPPD by PCR. A HindIII cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the 5' region of the promoter (in italics) is 5'- *ataagcttca tggagtcaaa -gattcaaata ga* -3' (SEQ ID No. 28), and that of the oligonucleotide which anneals onto the termination sequence (in italics) is 5'- *ataagcttgg ac-aatcagta aattgaacgg ag*-3' (SEQ ID No. 29). The resulting fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred as HindIII fragment from this PCR-Script vector into the correspondingly cut vector pBin19 (Bevan, 1984, Nucleic Acids Res. 12, 8711-8721).

Amend the paragraph on page 46, lines 1-17, as follows:

The 35S promoter, the transketolase transit peptide, the DOXS gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of

transcription was isolated from the plasmid pBinAR-TP-DOXS by PCR. An EcoRI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator sequence. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'- *atgaattcca tggagtcaaa gattcaaata ga* -3' (SEQ ID No. 30), and that of the oligonucleotide which anneals onto the terminator sequence (in italics) is 5'- *atgaattcgg acaatcagta aattgaacgg ag* -3' (SEQ ID No. 31). The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as EcoRI fragment into the correspondingly cut vector which already contained the HPPD sequence as described above.

Amend the paragraph on page 46, line 19, to page 47, line 2, as follows:

The 35S promoter, the GGPPOR gene and the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription was isolated from the plasmid pBinARHyg-GGPPOR by PCR. An XbaI cleavage site was attached in each case to the oligonucleotides for the promoter and the terminator. The sequence of the oligonucleotide which anneals onto the promoter (in italics) is 5'- *attctagaca*

tggagtcaaa -gattcaaata ga -3' (SEQ ID No. 32), and that of the oligonucleotide which anneals onto the terminator sequence (in *italics*) is 5'- *attctagagg acaa-tcagta aattgaacgg ag -3'* (SEQ ID No. 33). The fragment was purified using a Gene-Clean kit (Dianova GmbH, Hilden) and cloned in accordance with the manufacturer's information into the vector PCR-Script from Stratagene GmbH, Heidelberg. The correctness of the sequence was checked by sequencing. It was transferred from the PCR-Script vector as XbaI fragment into the correspondingly cut vector which already contained the HPPD and DOXS sequences as described above. The result was the construct pBinAR-DOXS-GGPPOR-HPPD (Figure 16), whose fragments have the following significance:

Fragment A (529 bp) comprises the 35S promoter of cauliflower mosaic virus (nucleotides 6909 to 7437 of cauliflower mosaic virus). Fragment B comprises the transit peptide of the plastidic transketolase. Fragment E comprises the DOXS gene. Fragment D comprises the polyadenylation signal of gene 3 of the T DNA of the Ti plasmid pTIACH5 (Gielen et al., 1984) for termination of transcription. Fragment F comprises the GGPPOR gene including the intrinsic plastid transit sequence.